Quantification of Ammonia Release from Fruit Fly (Diptera: Tephritidae) Attractants Using Infrared Spectroscopy

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ABSTRACT Ammonia is the primary attractant for tephritid fruit flies, and traps baited with synthetic attractants using ammonia formulations have been highly successful in capturing these pests. However, difficulties in quantifying release rates of ammonia have limited abilities to make comparisons among field tests of different species by using different formulations. Therefore, Fourier transform infrared (FTIR) spectroscopy was evaluated as a method to quantify ammonia from synthetic lures. Analysis of the headspace from commercial ammonium bicarbonate and ammonium acetate lures indicated that there is a large burst of ammonia liberated upon initial exposure of the lures, but after 5–7 d the release rates stabilize and remain steady for at least 60 d under laboratory conditions. During the period of steady release, FTIR static measurements showed an average of 0.12 and 0.21 μg of ammonia per 50-ml sample from ammonium bicarbonate and ammonium acetate lures, respectively. FTIR dynamic measurements from ammonium acetate lures indicated a steady release rate of $\approx$200 μg/h. Ammonia release rate from ammonium acetate lures could be reduced by decreasing the surface area of the release membrane, and the presence of crystal formations on the membrane seemed to decrease the longevity of the ammonium acetate lures.

KEY WORDS Tephritidae, ammonia quantification, Fourier transform spectroscopy, *Ceratitis capitata*, *Anastrepha ludens*

Tephritid fruit flies include several economically important agricultural pests worldwide. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Mexican fruit fly, *Anastrepha ludens* (Loew) are of considerable economic importance for fruit and vegetable production and export. The Mediterranean fruit fly is a major pest worldwide due to its distribution and large host range (Liquido et al. 1991). Because of the threat of introduction of *C. capitata* and *A. ludens* into areas of the world currently free from these pests, much emphasis has been placed on detection and eradication of these species. Effective insect detection systems are essential for preventing the establishment of exotic pests, and surveys for these flies are included in state and federal exotic pest detection programs in at least nine southern and southwestern U.S. states (Lance and Gates 1994). California, Texas, and Florida maintain large numbers of trimedlure-baited Jackson traps (Harris et al. 1971) for detection of male *C. capitata* and aqueous protein-baited McPhail traps (Newell 1936, McPhail 1939) for detection of male and female *C. capitata* and *A. ludens* (Gilbert et al. 1984).

In research comparing sugar-based fruit fly attractants, it was determined that protein impurities were responsible for fruit fly attraction when the odor of ammonia was noted from a number of test preparations (McPhail 1939). Like other tephritids, flies in the genus *Anastrepha* require protein meals for the completion of reproductive maturation (Bateman 1972), and ammonia is perceived as a volatile cue for protein-rich food sources (Bateman and Morton 1981). Electroantennographic (EAG) studies with the Caribbean fruit fly, *Anastrepha suspensa* (Loew), indicated that antennal response to ammonia varies with dose (Kendra et al. 2005b) and with age of female (Kendra et al. 2005a). Peak EAG response was measured from immature females with ovaries actively undergoing vitellogenesis, or deposition of yolk proteins (Kendra et al. 2006), and this coincides with the age of peak protein consumption reported by Landolt and Davis-Hernandez (1993).

Traps baited with liquid protein solutions or synthetic ammonia have been used for *Anastrepha* spp. as well as other tephritid fruit flies. Various formulations of synthetic ammonia have been used as baits for fruit flies, including ammonium acetate (Prokopy 1968, Moore 1969), ammonium carbonate (Liquido et al.

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1993), ammonium bicarbonate (Robacker and Warfield 1993), and ammonium hydroxide (Stills 1964, Boucher et al. 2001). Research on *A. ludens* and *C. capitata* found that additional components such as putrescine, methylamine, and trimethylamine enhance the efficacy of traps baited with synthetic ammonia (Robacker and Warfield 1993, Heath et al. 1995, Heath et al. 1997). Commercial formulations of ammonium acetate (Suterra LLC, Bend, OR) and ammonium bicarbonate (AgriSense-BCS Ltd., Mid Glamorgan, United Kingdom) are available as lures for use in fruit fly traps.

Critical to the investigation and development of improved lures is an accurate method to measure the amount of attractant released from the lures. Quantification of ammonia is difficult because of its volatility and corrosive nature. Previously reported techniques (Heath et al. 1995, Robacker and Bartelt 1996) result in considerable variation, which compromises quantification. Therefore, we investigated Fourier transform infrared (FTIR) spectroscopy as a method for quantification of ammonia from lures. FTIR spectroscopy uses the measurement of high-quality infrared spectra for chemical analysis and identification (Griffiths 1983). Briefly (Smith 1996), an incoming infrared beam is split into two optical beams, one of which reflects off a fixed flat mirror and one of which reflects off a flat mirror that travels a short distance. The two beams are recombined, resulting in a signal called an interferogram. The interferogram signal is then transmitted through or reflected off the substrate of interest, producing a signal that is uniquely characteristic of the substrate. This substrate signal is then decoded by Fourier transformation, which is calculated by computer, and results are presented as an infrared spectrum of absorbance (or transmittance) versus wavenumber. Attributes of FTIR spectroscopy include rapid scanning, high sensitivity, and high resolution, and it has been found useful in atmospheric monitoring, surface chemistry and on-line identification of chromatically separated materials (Griffiths 1983). This technology has diverse applications, including microbial and material analyses (Schmitt and Flemming 1998) and determination of mechanisms for gas-phase reactions (Niki et al. 1987).

We used FTIR spectroscopy to quantify ammonia from lures that were containerized (static measurement) and from samples where ammonia was entrained in a continuous flow of air (dynamic measurement). These methods were then used to compare ammonia release rates from ammonium acetate lures that had crystallized ammonium acetate on the release membrane (due to storage conditions) and from ammonium acetate lures with release membrane partially covered to reduce ammonia emission in field tests.

Materials and Methods

Instrumentation. The Fourier transform infrared spectrometer used was a Thermo Nicolet Magna 550II (Madison, WI) equipped with an enhanced mercury cadmium telluride (MCT-A) detector, and a KBr beamsplitter. A 2-m (200-ml) gas cell with ZnSe windows and a thermal jacket (Thermo Electron Corporation, Waltham, MA) was used for calibration and for static measurements. The detector was cooled with liquid N_2, and the gas cell was heated to 100°C, with a vacuum of −26.5 mm Hg. Air was purified ($CO_2 <$ 1 ppm) with a Whatman 75-52 FTIR pure gas generator (Tewksbury, MA) and a Hankison HIT-20 air dryer (Canonsburg, PA) to remove water. Spectra were obtained by subtracting background from 128 scans of the sample in the range between 4,000 and 700 cm^{-1}. The method consisted of quantitation of one band for ammonia in the region of 997–987 cm^{-1}. Collection parameters were as follows: time for collection, 01:09 min; resolution, 4; and data spacing, 1.928 cm^{-1}. Bench parameters were gain, 1; velocity, 3.1647; and aperture, 32. Spectra had zero filling, with Happ-Genzel Apodization and Mertz phase correction. Background was collected as a single beam by using the same parameters, and then it was subtracted from the ammonia samples.

Calibration Curves. FTIR calibration curves were generated by preparing known dilutions of anhydrous ammonia gas (99.99% pure) from a lecture bottle (Aldrich, Milwaukee, WI). Amounts of anhydrous ammonia used for standard preparation were 5, 10, 15, 20, 30, and 40 μl. These volumes were obtained using a gastight syringe (VICI Precision Sampling, Baton Rouge, LA) and subsequently introduced into a volumetric gastight cell and diluted to 1 liter. An aliquot of 50 ml was obtained with a larger gastight syringe and injected into the FTIR gas cell, maintained at 100°C with a vacuum of −26.5 mm Hg. A processing method was created to analyze bands in the 997–987 cm^{-1} region for ammonia. Absorbance values were obtained based on peak area, and absorbance versus concentration was used to obtain a calibration curve.

Static Measurements. Static measurements of ammonia were obtained from commercial ammonium bicarbonate lures and ammonium acetate lures. A lure was placed in a volatile collection chamber, where it was purged for 1 h at room temperature (24°C) and ambient pressure by using a purified air flow of 1 liter/min. With a gastight syringe a 50-ml aliquot was taken through an open port in the volatile collection chamber and injected into the evacuated FTIR cell. FTIR analysis was performed as described above, and results were processed using TQ Analyst, version 6 (Thermo Electron Corporation). Quantities (in micrograms) of ammonia were calculated for each sample from the FTIR calibration curve. Final quantitative determinations were mean values based upon five measurements. Lures were placed in a hood at ambient room temperature and humidity when not sampled. Ammonia was quantified as micrograms per 50 ml per minute, which was converted to release rate of micrograms per liter per hour by multiplying by 1,200.

Dynamic Measurements. To compare results from measurements made in the static mode, release rates from ammonium acetate lures were also determined using a dynamic mode. The lures were placed in 500-ml glass chambers containing an inlet and outlet

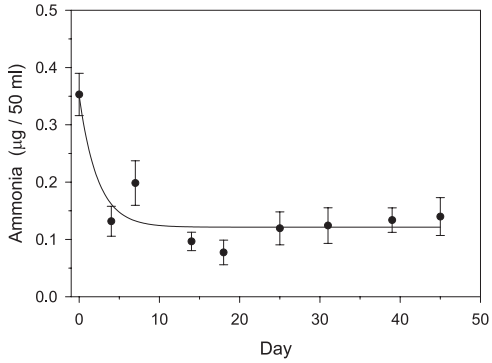

Fig. 1. Amount of ammonia (mean ± SE) released over time from commercial ammonium bicarbonate lures ($n = 5$). Determinations made using FTIR static method.

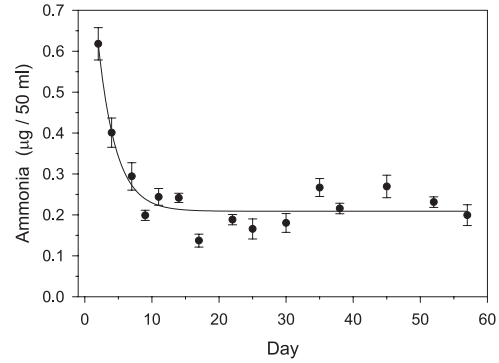

Fig. 2. Amount of ammonia (mean ± SE) released over time from commercial ammonium acetate lures ($n = 5$). Determinations made using FTIR static method.

port. All connections to the chamber were done using Teflon tubing (Fisher, Hampton, NH). Carrier gas was from the same source as that used for the FTIR and was used to purge the ammonium samples to the FTIR gas cell. Flow was metered using a flow meter (Aalborg, Monsey, NY) at 1 liter/min. After the gas cell was purged for 5 volumes, a measurement was taken. Effluent from the gas cell was vented into a hood. Release rate determinations were mean values based upon measurements from five lures. Lures were placed in a hood at room temperature and humidity when not sampled. Ammonia was quantified as micrograms per 200 ml per minute, which was converted to release rate of micrograms per liter per hour by multiplying by 300.

Newly obtained ammonium acetate lures typically have no apparent ammonium acetate crystals; however sometimes older lures will have some crystals present on the surface of the release membrane. It is not known whether presence of crystallized ammonium acetate affects ammonia release rate or lure longevity. Therefore, release rate was determined over time from two different lots of ammonium acetate lures: one lot with crystals present and one lot with no noticeable crystals. Five lures were tested per lot.

Finally, it has been shown that a reduction in membrane surface area will decrease ammonia release (Heath et al. 1995). For ongoing field studies with ammonium acetate lures, metallic tape (United Tape Co., Cummings, GA) is used to cover half the membrane surface to provide reduced release lures. Therefore, release rate determinations were made from five standard lures (full lure, no tape added) and five half lures (50% surface area reduced with tape). All lures were placed in a hood and held at ambient room temperature and humidity when not sampled.

Statistical Analysis. Regression analysis was used to describe the relationships between ammonia release rate and time (SigmaPlot 8.0, SPSS Inc., Chicago, IL). Several regression models were tested including second and third order polynomial, power functions, and exponential decay.

Results

FTIR analysis of the anhydrous ammonia standards resulted in a calibration curve with a strong linear regression ($r^2 = 0.995$, $y = 0.0727x + 0.111$; x is ammonia concentration and y is peak absorbance area). FTIR analysis of the headspace from commercial ammonium bicarbonate and ammonium acetate lures showed that there was a large burst of ammonia liberated upon initial opening of the packaged lures, but in most cases the release rates stabilized within 5–7 d of exposure (Figs. 1–4). FTIR static measurements from the ammonium bicarbonate lure indicated that ammonia release over the 45-d sampling period was best fit by regression with an exponential decay model ($y = 0.1214 + 0.2294e^{-0.4340x}$; $R^2 = 0.8447$; Fig. 1). After stabilization was reached, the mean quantity of ammonia per 50 ml of headspace was 0.1214 μg, which converts to a release rate of 145.68 μg/h from the ammonium bicarbonate lure. Similar analysis in the static mode found that ammonia release from the ammonium acetate lure over a 57-d period also was best fit by an exponential decay model ($y = 0.2089 + 0.8627e^{-0.3729x}$; $R^2 = 0.9004$; Fig. 2). Once stabilized, the ammonium acetate lure emitted mean ammonia values of 0.2089 μg/50 ml headspace, or 250.68 μg/h.

The FTIR dynamic method was used to compare ammonia release from ammonium acetate lures with and without crystal deposits on the release membrane. As with the static method, data obtained with the dynamic method were best fit by regression with an exponential decay model, both for lures without crystals ($y = 190.3402 + 676.8942e^{-0.3211x}$; $R^2 = 0.8934$; Fig. 3) and for those with crystals ($y = 222.0165 + 2023.4937e^{-0.3269x}$; $R^2 = 0.9255$; Fig. 4). Once the lures stabilized, the average ammonia release rate over the 74 d of sampling was 190.34 μg/h for lures without crystals and slightly higher, at 222.02 μg/h, for lures with crystals. However, the initial burst of ammonia measured after 4 d was twice as high in lures with crystals than in lures without crystals (771.2 ± 93.90 versus 379.1 ± 15.65 μg/h, respectively). Lures with crystals continued to release more ammonia during the 8–54-d sampling period (270.0 ± 9.44 μg/h with

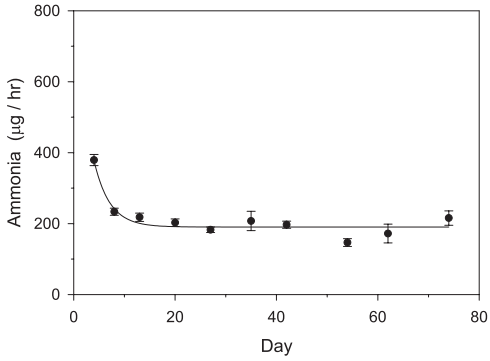

Fig. 3. Ammonia release rate (mean ± SE) over time from a lot of ammonium acetate lures without crystals (n = 5). Determinations made using FTIR dynamic method.

crystals versus 198.2 ± 6.52 µg/h without crystals), but then the release rate declined (Fig. 4). By the final two measurements (62 and 74 d) the trend was reversed, and lures with crystals released less ammonia than lures without crystals (143.1 ± 31.01 versus 193.8 ± 17.31 µg/h, respectively). These results suggest that crystal formation may increase ammonia release initially, but decrease release rate with extended periods of exposure, somewhat reducing lure longevity.

In the study, evaluating the effect of surface area on ammonia release rate, regression with exponential decay again gave the best fit between release rate and time of exposure (full lure: $y = 197.1985 + 542.5597e^{-0.1007x}$; $R^2 = 0.8893$; half lure: $y = 145.6656 + 165.4266e^{-0.1150x}$; $R^2 = 0.8575$; Fig. 5). Unlike the previous studies in which the release rate stabilized within 5–7 d, the lot of lures tested in this study showed a steady decline in rate over a several week period. Reducing the membrane surface area by 50% resulted in a reduction in the amount of ammonia released by the lure. Initially the release rate was reduced by approximately half until day 14, after which the percentage of reduction declined to ≈30% by the final measurement at 51 d. After the first measurement, taken after a 3-d exposure, the ammonia release rate

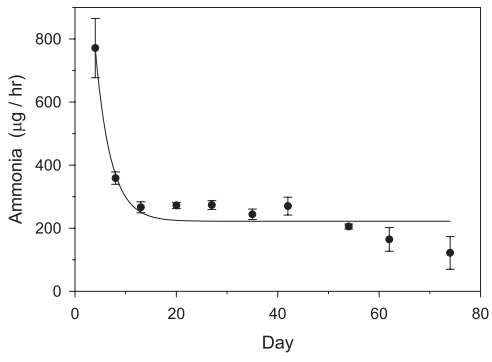

Fig. 4. Ammonia release rate (mean ± SE) over time from a different lot of ammonium acetate lures containing visible crystals on the release membrane (n = 5). Determinations made using FTIR dynamic method.

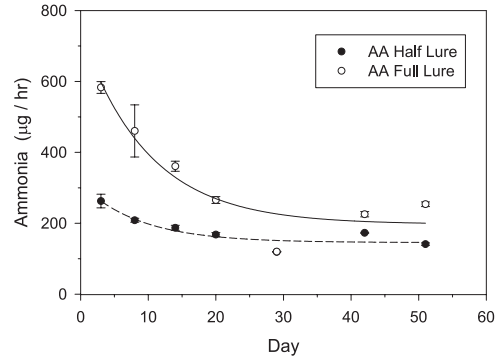

Fig. 5. Ammonia release rate (mean ± SE) over time from ammonium acetate lures with membrane fully exposed (full lure, solid line, open circle, n = 5) and with half the membrane surface covered with tape (half lure, dashed line, solid circle, n = 5). Determinations made using FTIR dynamic method.

averaged 166.2 ± 5.69 µg/h from the half lure and 281.0 ± 22.96 µg/h from the full lure.

Overall, the FTIR dynamic method was used to evaluate release rate from three separate lots of ammonium acetate lures: lures without crystals (Fig. 3), lures with crystals (Fig. 4), and full and half lures in the surface area study (Fig. 5). The mean steady release rate of ammonia from full lures tested was determined (from regression analysis) to be 203.2 ± 9.62 µg/h.

Discussion

FTIR provided an accurate, precise, and rapid method for quantification of ammonia compared with previous results by using an ammonia-specific ion-selective electrochemical probe (Heath et al. 1995, and references therein). For ammonia determinations with an ion-selective probe, the test substrate is placed in an Erlenmeyer flask, and the flask is purged for 1 h with an airflow of 1 liter/min. Volatiles are directed to a sparge system that consists of a gas dispersion tube placed in a graduated cylinder containing a 0.05 N HCl solution. After collection, the ionic strength of the sample solution is adjusted to pH 9.0 by using NaOH/0.05 M disodium EDTA/10% methanol containing a color indicator for pH. A standard ammonium calibration curve has to be prepared each day an analysis is done. Using FTIR, no sample preparation is needed other than dilution. Variance-to-mean ratios can be used as an indication of precision (Levinson and Tumbelty 1997), and ratios of 11–42% were obtained using the ion-selective probe (Heath et al. 1995), whereas ratios of 3–9% were obtained using FTIR.

Robacker and Bartelt (1996) used solid-phase microextraction (SPME) to quantify ammonia. For this technique, SPME fibers were immersed in solutions of test substrates. Test substrates were prepared from six concentrations of ammonium carbonate added to 2% phosporic acid (for calibration curves) or from lures that were placed in closed vessels that contained 2% phosphoric acid for 24 h at 35°C to trap volatile chem-

icals (for release rate estimations). Subsequently, the pH was adjusted with aqueous sodium hydroxide either immediately (for calibration curves) or after lure removal. Variance-to-mean ratios were similar to those obtained with FTIR, with ratios of 4–5%. However, the authors point out that errors inherent in the SPME/GC technique due to difficulties with and time constraints in generating the calibration curves resulted in errors in accuracy of ± 50%. In addition to problems with accuracy, the correlation between ammonia trapped in solution versus ammonia released as a vapor is not known.

The strong linear relationship between amount of ammonia and absorbance obtained by FTIR analysis was expected. Admittedly, each candidate test substrate must be evaluated to ensure that no interfering absorption occurs in the 997–987 cm^{-1} region. A constant background of absorbance in this region is readily addressed by background subtraction if the dynamic range of the detector is sufficient.

The long-term goal of this research is to develop improved synthetic lures for capturing economically important fruit flies. A critical component is the effect of release rate of ammonia on the attraction of fruit flies (Heath et al. 1995), and systems to quantify this type of corrosive and volatile gas continue to provide analytical challenges. Recent studies by Kendra et al. (2005 a,b) have shown that quantitative amounts of ammonia can be used to examine the physiological basis for attraction to food-based lures by comparing EAG responses to individual putative attractants such as ammonia, or combinations of attractants, within and among tephritid species. Use of saturated vapor enables the collection and delivery of precise amounts of ammonia and other putative attractants for EAG determinations. Additionally, the method described can provide lure manufactures a facile system to make improvements in longevity of ammonia formulations for use in monitoring exotic fruit fly species and other insects that respond to ammonia-based lures.

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